

## Morphology and Anatomy of Winter Bud of *Pteridophyllum racemosum* (*Pteridophyllaceae*)

Yasuhiko ENDO<sup>a</sup>, \* Jun SAITO<sup>a</sup> and Keiichi OONO<sup>b</sup>

<sup>a</sup> College of Science, Ibaraki University, Mito, 310-8512 JAPAN

<sup>b</sup> Natural Science Museum and Institute, Chiba, 955-2, Aoba-cho, Chuo-ku, Chiba, 260-8682 JAPAN

\*Corresponding author: endoy@mx.ibaraki.ac.jp

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*Pteridophyllum racemosum* (*Pteridophyllaceae*), an evergreen herb endemic to Japan, produces winter buds on the ground surface. Buds consist of three to four bud scales, young leaves, axillary buds, and a young inflorescence. Young leaves and the young inflorescence curve downward in the bud. Young leaves show circinate vernation. Bud scale tissues are composed of an epidermis, cortex, and a vascular bundle. The cortex is characterized by relatively wide intercellular spaces and deposition of starch grains in the cells.

**Key words:** Circinate vernation, intercellular space, phyllotaxis, prophyll, *Pteridophyllum racemosum*, winter bud.

*Pteridophyllum racemosum* Siebold & Zucc. (*Pteridophyllaceae*) is an evergreen herb endemic to Japan where it is distributed in the mountainous and subalpine areas of the central and northern parts of Honshu (Mizuno et al. 1975, Oono 1999, Akiyama 2006). Leaves are pectinate, radical, and form a rosette; the inflorescence is a raceme and stands at the center of the rosette; flowers have 2 sepals, 4 petals, 4 stamens, and 1 pistil and open from June to August (Ohwi 1965, 1982, Akiyama 2006). After flowering, winter buds grow approximately at the center of the rosette, on the ground surface (Kimura 1970a, 1970b, Mizuno et al. 1975). To understand the resistance mechanism by which aboveground winter buds resist environmental stresses, especially freezing, we studied the morphological features and internal structures of winter buds. This is the first report on the

morphology and anatomy of *P. racemosum* winter buds. We could also get some information about vernation and phyllotaxis of the species by the present analysis of the winter bud internal structures.

### Materials and Methods

Winter buds were fixed in FAA (50% aqueous ethanol : formaldehyde : acetic acid = 9 : 0.5 : 0.5). The outer morphological features were observed using a dissecting microscope and were sketched or photographed. Internal structures were examined by cutting the buds transversely into 4 to 7 µm thick sections using a manual rotary microtome. Sections were stained using a combination of Heidenhain's haematoxylin, safranin, and Fast Green FCF, before observation under a light microscope.

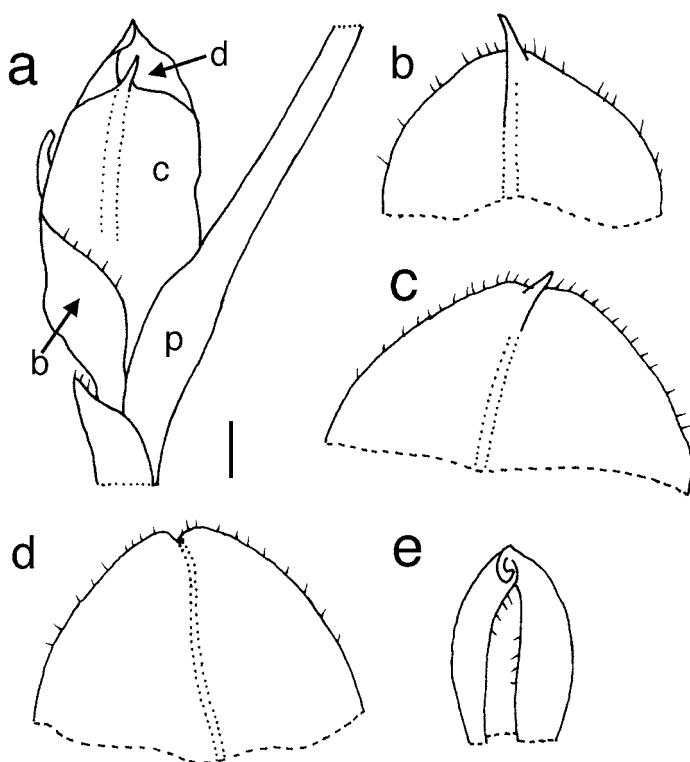


Fig. 1. Outer morphology of winter bud of *Pteridophyllum racemosum*, collected at Mt. Kinunuma-yama, Tochigi Pref. on 22 Oct. 2009. a. Entire bud. b–e. Bud scales. The position of each bud scale is shown in Fig. 1a. e. An innermost bud scale covered by other three bud scales (b–d). b–d. Abaxial side. e. Adaxial side. p. Petiole. Scale bar = 1mm.

## Results and Discussion

### Morphological features

The outermost edge of buds was composed of three to four bud scales (Fig. 1). Some bud scales had a small leaf blade at their apex. Therefore, bud scales appeared to originate from a petiole. Bud scales were semi-circular, fringed by short hairs, and distinguishable from the petioles of foliage leaves (Fig. 1).

Only the outermost one or two bud scales subtended an axillary bud (Fig. 2). Inside the scales, there were four to seven young leaves and a young inflorescence (Figs. 2, 6, 7). When the helically arranged bud scales (s0–s3 in Fig. 2) appeared in a counterclockwise order, the basal young leaves (y4–y6) in the bud

appeared in the same order. On the other hand, sometimes the apical young leaves (y'7–y'10) appeared in a clockwise order. Such change of leaf arrangement has been reported to appear between the main axis and the lateral branch (Tucker 1963, Kumazawa 1960, 1963a, 1963b, 1967, 1979, Kumazawa and Kumazawa 1970, Gómez-Campo 1970, Iwamoto et al. 2005). Therefore, we presume that the apical leaves (y'7–y'10) would be originated from the axillary bud (of which the subtending leaf would be y6) (Fig. 2). This means that the inflorescence originated from an apical bud, and that the branching of the stem in the bud would be sympodial.

There were two or three axillary buds in a

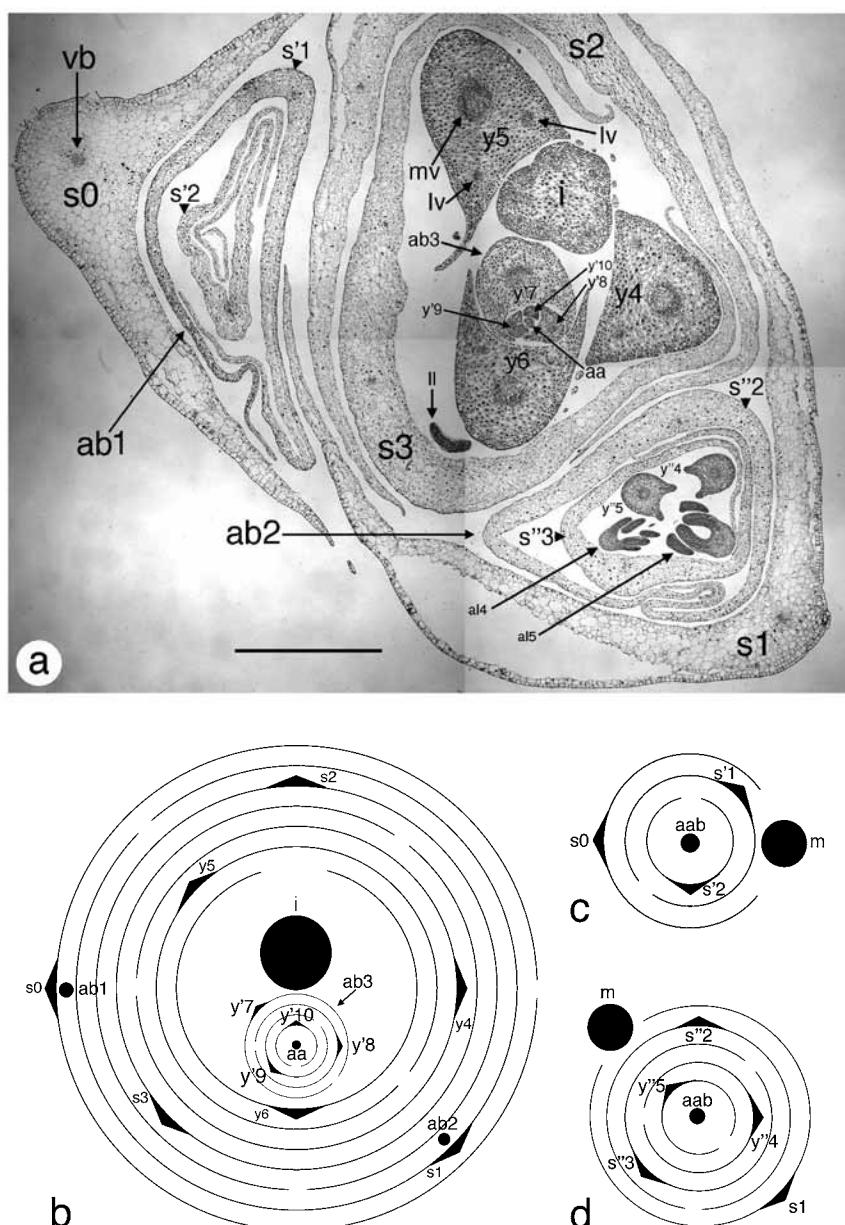


Fig. 2. Transverse section of the basal part of winter bud, including reproductive shoot, of *Pteridophyllum racemosum*, collected at Misato-machi, Daisen-shi, Akita Pref. on 18 Oct. 2010 (Endo 3652, TUS), showing an arrangement of bud scales, young leaves, inflorescence, and axillary buds. a. Entire section. b. A schematic diagram of entire section. c. A schematic diagram of axillary bud "ab1". d. A schematic diagram of axillary bud "ab2". aa. Apex of axillary bud. aab. Axis of axillary bud. ab. Axillary bud. al. Apical part of leaf. i. Inflorescence. ll. Leaf lobe. lv. Lateral vein. m. Main axis of winter bud. mv. Midvein. s, s', s''. Bud scale. vb. Vascular bundle. y, y', y''. Young leaf. Closed triangle indicates position of the midvein of bud scale or young leaf. The numbers alongside the abbreviations indicate their relative position in the winter bud, i.e., higher numbers indicate positions toward the center of the bud for the same organ type. Scale bar = 1 mm.

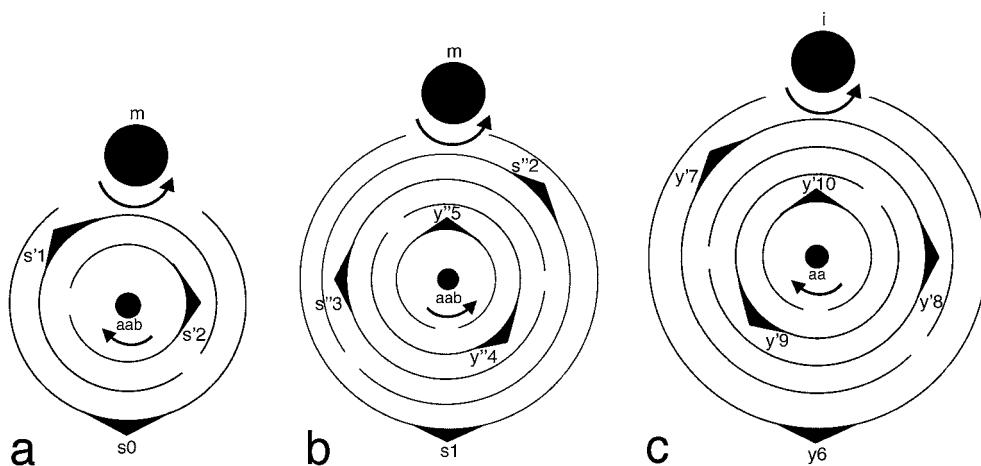


Fig. 3. Schematic diagrams of axillary buds of a winter bud of *Pteridophyllum racemosum* in Fig. 2, indicating the direction of the phyllotactic helix. a. Axillary bud "ab1" in Fig. 2. b. Axillary bud "ab2" in Fig. 2. c. Axillary bud "ab3" in Fig. 2. aa. Apex of axillary bud. aab. Axis of axillary bud. i. Inflorescence. m. Main axis of winter bud. s', s''. Bud scale. y', y''. Young leaf. Closed triangle indicates position of the midvein of bud scale or young leaf. The numbers alongside the abbreviations indicate their relative position in the winter bud, i.e., higher numbers indicate positions toward the center of the bud for the same organ type. The arrows indicate the direction of phyllotactic helix, i.e., long arrow for foliar organs of main axis and short one for those of axillary bud.

winter bud of *P. racemosum* (ab1, ab2, and ab3 in Figs. 2a–d). These axillary buds consisted of young leaves and/or bud scales (Figs. 2a–d). The two most basal foliar organs of the axillary buds have been called prophylls (Blaser 1944, Kumazawa 1979). Therefore, the pairs of young leaves, s'1 and s''2 of the axillary bud 'ab1', s'1 and s''2 of bud 'ab2', and y'7 and y'8 of bud 'ab3', would be prophylls (Fig. 2). The divergent angle between the two prophylls, y'7 and y'8, was ca. 135° (Figs. 2a, b). In the two prophylls, the more basal one (y'7 in Figs. 2a, b) and the subtending leaf of the axillary bud (y6 in Figs. 2a, b) show a divergent angle 'ca. 135°' between them (Figs. 2a, b). On the other hand, dicotyledonous plants have been reported to show the divergent angle 'ca. 180°' between the prophylls as typical (Kumazawa 1979). These prophylls usually position in a direction perpendicular to the subtending leaf of the axillary shoot (Kumazawa 1979).

In monocotyledonous plants, the prophyll is usually only one and positioned at the opposite side of the subtending leaf (Kumazawa 1979). However, the arrangement of prophylls in *P. racemosum* may be specialized among seed plants. To understand the systematic implications of the specialized arrangement of the prophylls, comparative morphology on the related species of *P. racemosum* would be needed.

In a winter bud of *P. racemosum*, foliar organs of some axillary buds appeared in a clockwise order (Figs. 3a, c), but those of the other axillary buds appeared in a counterclockwise order (Fig. 3b). This difference of the direction of the phyllotactic helix would be shown as the difference of the relative position between the most basal prophyll and the subtending leaf of the axillary bud, i.e., when the most basal prophyll appeared in clockwise order (s'1 in Fig. 3a, and y'7 in Fig. 3c) against the subtending leaf of the axillary bud (s0 in Fig.

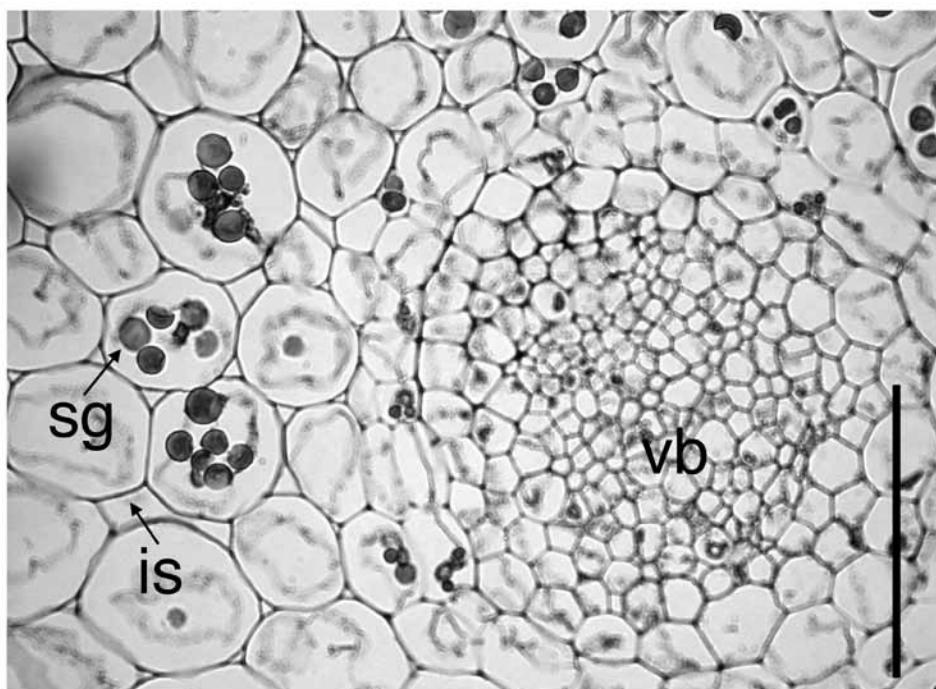


Fig. 4. Transverse section of a part of *Pteridophyllum racemosum* winter bud scale near the vascular bundle, collected at Mt. Taihei-zan, Akita Pref. on 11 Aug. 2007 (Endo 3569, TUS), showing intracellular starch grains and relatively wide intercellular spaces. is. Intercellular space. sg. Starch grain. vb. Vascular bundle. Scale bar = 0.1 mm.

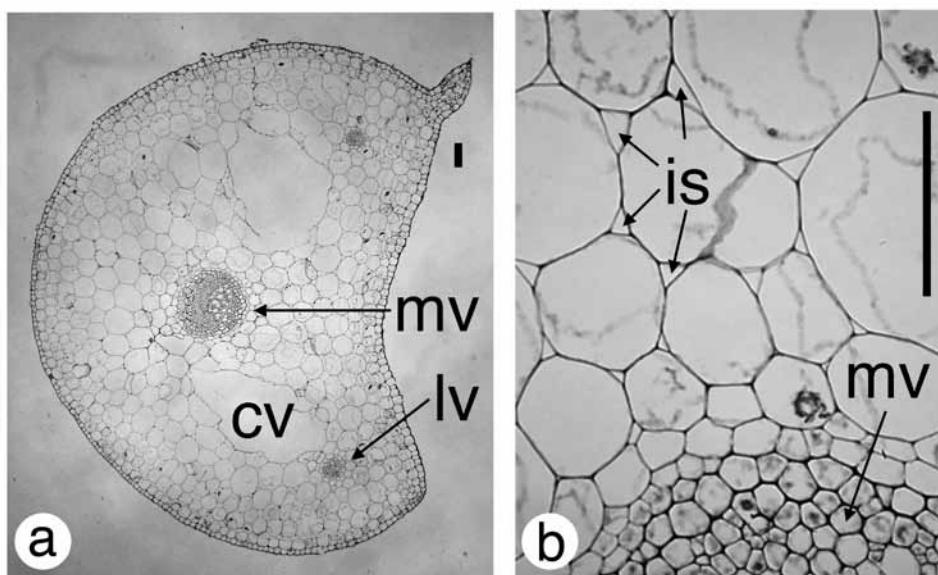


Fig. 5. Transverse section of petiole of *Pteridophyllum racemosum*, collected at Mt. Ontake-san, Gifu Pref. on 29 Oct. 2009 (Endo 3640, TUS), showing three vascular bundles and relatively wide intercellular spaces. a. A whole part. b. Enlargement of the cortex and vascular bundle of the petiole. cv. Cavity. is. Intercellular space. lv. Lateral vein. mv. Midvein. Scale bar = 0.1 mm.

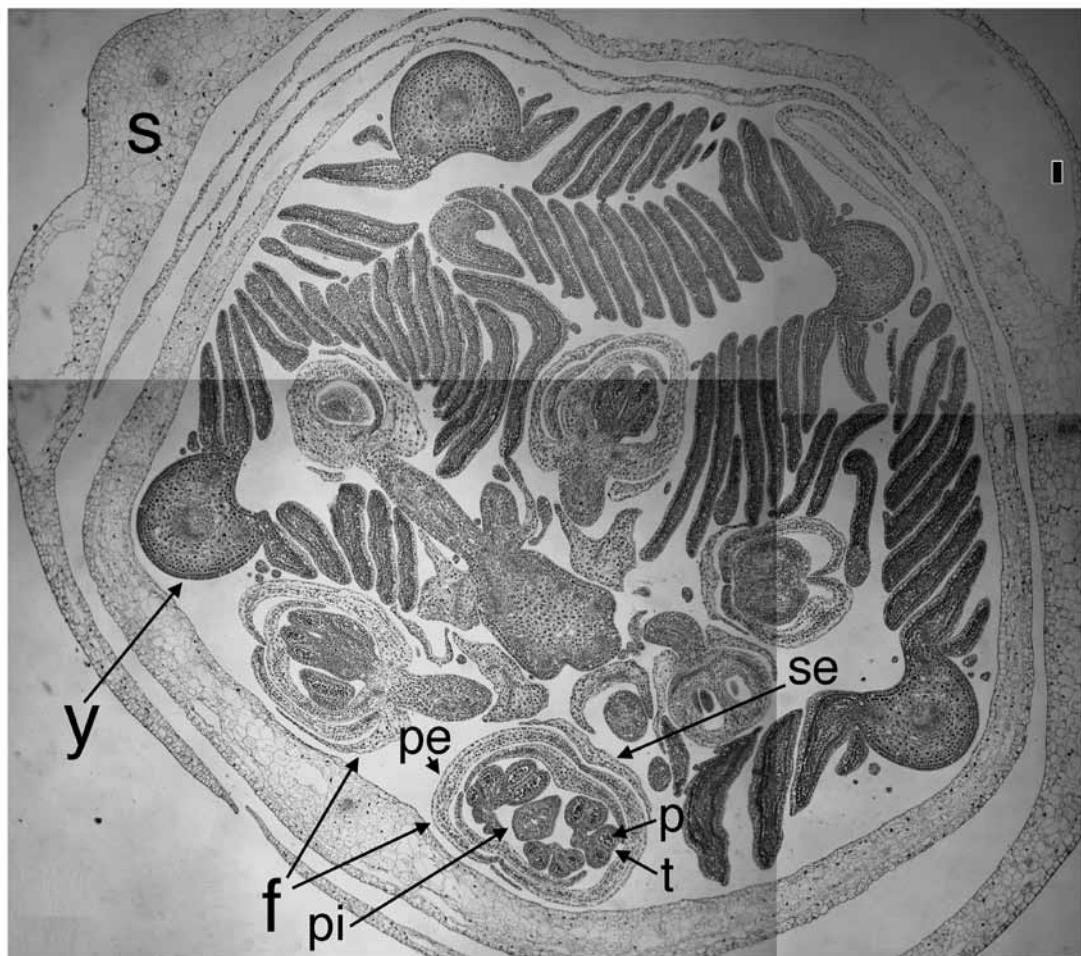


Fig. 6. Transverse section of the apical part of a winter bud of *Pteridophyllum racemosum*, collected at Mt. Ontakesan, Gifu Pref. on 29 Oct. 2009 (Endo 3640, TUS), showing the positions of young leaves and inflorescence, and the internal structure of flower buds. f. Flower bud. p. Pollen mother cell. pe. Petal. pi. Pistil. s. Bud scale. se. Sepal. t. Theca. y. Young leaf. Scale bar = 0.1 mm.

3a, and y<sub>6</sub> in Fig. 3c), other foliar organs of the bud appeared in the same order successively (s'2 in Fig. 3a, and y'8, y'9, and y'10 in Fig. 3c); when the most basal prophyll appeared in counterclockwise order (s''2 in Fig. 3b) against the subtending leaf of the axillary bud (s1 in Fig. 3b), other foliar organs of the bud appeared in counterclockwise order successively (s''3, y''4, and y''5 in Fig. 3b). Such variation of the position of the most basal prophyll relative to the subtending leaf among the axillary shoots in one individual has been reported in many dicotyledonous species (Kumazawa 1960,

1963a, 1963b, 1967, 1979, Kumazawa and Kumazawa 1970). Therefore, a variation of the direction of the phyllotactic helix of axillary buds, observed in a winter bud of *P. racemosum*, would not be unusual in Dicotyledons. How the position of the most basal prophyll relative to the subtending leaf is determined is still in question.

The flower buds showed two sepals, four petals, a pistil and four stamens with thecae (Fig. 6).

The phyllotaxis was determined to be spiral from the bud structure (Figs. 2a, 2b). The divergence may be 3/8 (Figs. 2a, b). The young

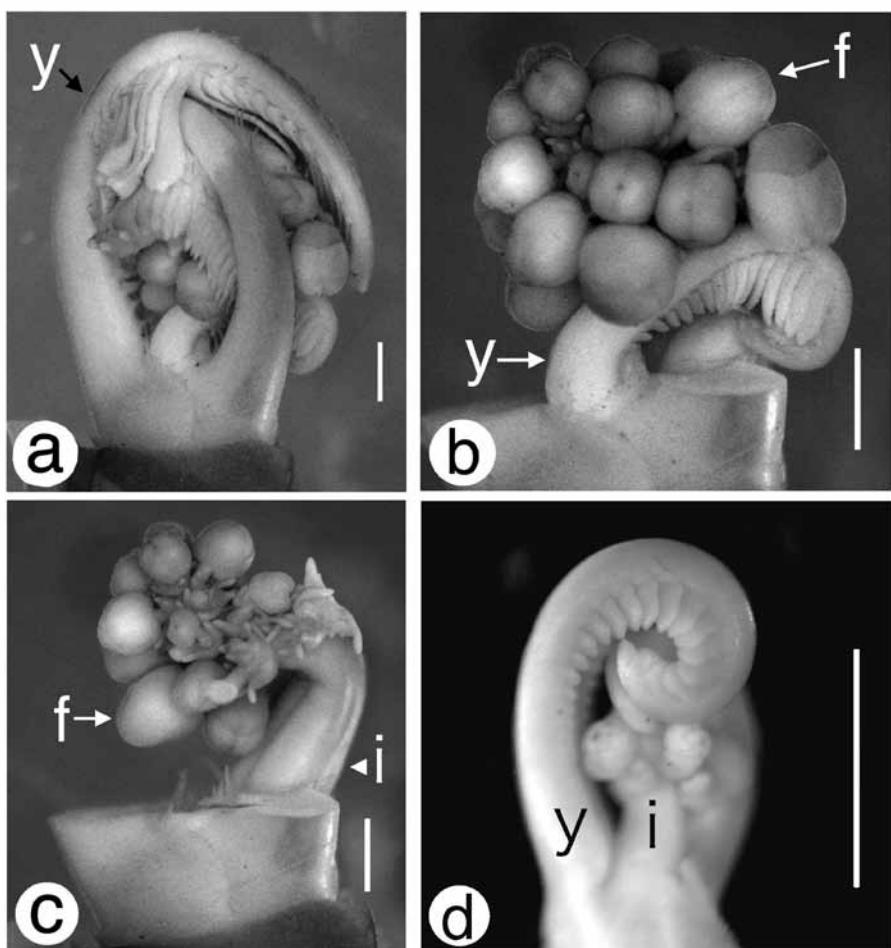


Fig. 7. Outer morphology of winter bud of *Pteridophyllum racemosum* showing curving young leaves and a curving inflorescence. a. Bud scales were cut off to show a young inflorescence covered by curving young leaves. b. Bud scales and two outer young leaves were cut off to show a curving young leaf. c. Bud scales, young leaves, and several basal flower buds were cut off to show a curving young inflorescence. d. Bud scales were cut off to show a young leaf with circinate vernation. a—c, collected at Mt. Ontake-san, Gifu Prefecture in Oct. 29, 2009 (Endo 3640, TUS). d, collected at Mt. Taihei-zan, Akita Prefecture in Aug. 11, 2007 (Endo 3569, TUS). f. Flower bud. i. Inflorescence. y. Young leaf. Scale bar = 1 mm.

leaves and an inflorescence curved from the tips downward in the winter buds (Figs. 7a–c). The young leaves showed circinate vernation (Fig. 7d). Such vernation has been reported in *Droceraceae* in angiosperms (Kenneth et al. 2002), and may be rare in angiosperms. To know whether circinate vernation is an apomorphy or a plesiomorphy for *Pteridophyllaceae*, we need to know distribution of the vernation type among

the families related to *Pteridophyllaceae*.

#### Anatomical features

Scale tissues of *Pteridophyllum racemosum* were composed of an epidermis, a cortex, and a vascular bundle (Figs. 2a, 3).

The scale cortex was composed of parenchymatous cells, with intercellular spaces (Fig. 4). The cortex of the petiole also had

intercellular spaces (Fig. 5). The intercellular spaces might help intercellular freezing of water and prevent intracellular freezing, which destroys intracellular structures (Sakai and Larcher 1987). Therefore, the presence of intercellular spaces in winter buds may be a mechanism for resisting lower temperatures during the winter. In further examination, the mechanism has to be confirmed experimentally in *P. racemosum*.

Each scale had only one vascular bundle (Fig. 2a), but the leaf petiole had three vascular bundles, i.e., one mid-vein and two lateral veins (Figs. 2a, 5a). Therefore, scales could be distinguished from leaf petioles based on their internal structures. We suggest that the winter bud of *P. racemosum* would be classified as a type C winter bud, i.e., having perfectly differentiated scales, as described by Yoshie and Yoshida (1989).

The cortex of bud scales contained starch grains (Fig. 4). Furthermore, the leaves of *P. racemosum* also contain starch grains in winter and are considered to act as storage organs (Kimura 1970a). Thus, bud scales would share a storage function with the winter leaves.

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### 遠藤泰彦<sup>a</sup>, 斎藤 潤<sup>a</sup>, 大野啓一<sup>b</sup>: オサバグサの冬芽の形態と構造

日本固有で、山地帯から亜高山帯に生育する常緑草本オサバグサ *Pteridophyllum racemosum* (オサバグサ科) は地表面に冬芽を形成する。冬芽の最も外側には、葉柄から分化したと考えられる 3 枚から 4 枚の芽鱗がある。芽鱗は表皮、皮層、そして 1 本の維管束より成り、3 本の維管束が認められる葉柄とは明確に異なっている。芽鱗の内側に、渦巻き状をした幼葉と湾曲した花序が配置されている。このような渦巻き状の幼葉重疊法は被子植物では稀である。芽鱗の皮層は比較的広い細胞間隙と細胞内のデンプン粒によって特徴づけられる。ここで見られる広い細胞間隙は、冬期の低温による細胞内凍結を避ける構造と考えられる。花序は頂芽由来であり、茎は仮軸分枝している。葉序は螺旋葉序で、開度は 3/8 である。

一般に単子葉類を除く種子植物では、側枝最基部に

前葉と呼ばれる 2 枚の葉をつけることが知られている。この 2 枚の葉は、蓋葉の左右の方向に着くことから、側生前葉と呼ばれ、対生する (開度 1/2)。一方、本研究での冬芽の観察結果、オサバグサの側枝では側生前葉に相当する 2 枚の葉の開度は 3/8 であり、この点で特徴的である。

本研究で認められた被子植物でも稀なオサバグサの特徴、つまり (1) 渦巻き状の幼葉重疊法、(2) 開度 3/8 の前葉、の分類学的意義については、オサバグサに近縁な分類群の相当する特徴と比較検討することにより明らかにする必要がある。

(<sup>a</sup>茨城大学理学部,

<sup>b</sup>千葉県立中央博物館)